Low pH as the Limiting Factor for Survival of the Mosquito Larvivorous Fish, *Poecilia reticulatus*, in Impounded Sugar Mill Wastewater

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ABSTRACT

Acidity below pH 5.5 caused asphyxiation of *P. reticulatus* despite adequate dissolved oxygen and low levels of carbon dioxide in sugar mill wastewater. Clogging of gills with precipitated iron when acidity fell below pH 5.5 is suspected of causing asphyxiation. No fish survived below pH 4.8 even at high DO and low CO_2 levels. Survival of 60% occurred between pH 4.9-5.3, while all fish survived above pH 5.5 even at low DO (0.3 ppm) and high CO_2 (250 ppm) levels. Primary cause of low pH and high CO_2 levels was biological decomposition of suspended microscopic particles of sugar cane stalks. Major source of this material is from sludge precipitated by lime during clarification of cane juice. Separation of this sludge may increase pH in impounded wastewater to tolerable levels for fish survival.

Suburbanization close to sugar mills and government antipollution regulations have recently caused increased mosquito control problems for sugar plantations in Hawaii. The building of residential communities within the 3.5 mile flight range of *Culex quinquefasciatus* Say (U.S. Navy 1963) has resulted in stringent control measures for mosquitoes breeding in canefields irrigated with sugar mill wastewater. Mosquito breeding potential in mill wastewater was also increased when government antipollution regulations prohibited the discharge of excess sugar mill wastewater into the sea. The effect of this prohibition was an increase in mosquito breeding sites caused by the construction of large impoundment basins for wastewater storage and the inundation of the limited acreage of irrigated canefields close to mills.

The difficulty in keeping mosquito populations below complaint levels can be appreciated when one considers that mosquito breeding in as little as a quarter acre of wastewater-flooded canefield has caused mosquito nuisance complaints from neighboring communities. Despite these difficulties, most sugar companies have managed to keep mosquito breeding below complaint levels, but only at considerable expense. Biological control as a means of reducing larviciding costs was considered when I observed *P. reticulatus* surviving in certain areas of impounded wastewater during three of five seasons of mill operations at Oahu Sugar Company, Waipahu, Oahu.

This study was conducted to: (1) discover the limiting factors of fish survival in sugar mill wastewater; (2) find the source of these limiting factors; and (3) determine whether these limiting factors can be reduced to tolerable levels for fish survival.

MATERIALS AND METHODS

Sugar mill wastewater analyses and field observations were done on Waipio Peninsula, Oahu. The peninsula has approximetely 750 acres of canefields and is the discharge point for wastewater from Oahu Sugar Company's mill located a mile away in Waipahu. About eight million gallons of wastewater is discharged per day into the peninsula for cane irrigation. Daywater is used directly for irrigation while nightwater is stored in large impoundment basins for controlled release during the day.

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Limiting Factors

Limiting factors of fish survival were determined by: (1) analyzing wastewater to find potential toxicants; and (2) bioassaying each potential toxicant.

Mill Wastewater Analyses. Mill wastewater was analyzed for temperature, salinity, pH, turbidity, dissolved oxygen (DO), carbon dioxide (CO_2), sulfides, ammonia, and iron. Five samples were taken randomly during June and July at three locations: (1) the mill wastewater outfall; (2) a 25-acre wastewater impoundment basin; and (3) the end of a mile-long wastewater canal that removed overflow from impoundment basins to canefields.

Commercial colorimeter test kits were used to sample sulfides, ammonia, and iron. For convenience, a smaller 20 ml sample of the Standard Titrimetric Method (APHA 1975) was used to sample free CO_2. Comparison of 20 ml samples with normal 100 ml samples for CO_2 showed an error of 4.0% higher readings for the 20 ml samples. Surface samples for DO were taken by allowing surface water to flow with minimal agitation into a beaker held below the water to its rim. Turbidity was determined with a Secchi Disk.

Bioassay of DO, CO_2, and pH. Varying concentrations of DO, CO_2, and pH in different combinations were used to determine which of these potential toxicants caused fish mortality. Aged wastewater, past the biological decomposition stage, was used for control samples and as stock for various combinations of CO_2, DO, and pH concentrations. High and low concentrations were: pH, 6.1–4.7; CO_2, 25–80 ppm; and DO, 14.0–0.4 ppm.

CO_2 and O_2 were bubbled into the water to attain desired concentrations. Adjustments of CO_2 concentrations were made before changes in pH because CO_2 could not be measured after the addition of a mineral acid. The increase of CO_2 from carbonates in acidified water was considered minimal because the water had already undergone pH 4.7 during the biological decomposition stage. Acidity was altered with phosphoric acid. Five male *P. reticulatus* were placed in each 500 ml sample and observed for 24 hours. *P. reticulatus* used in bioassays were collected from wastewater basins and kept in aquariums containing wastewater. Only adult males were used for testing because of their uniform size.

Bioassay of Low pH in Wastewater. Whether low pH was solely responsible for fish mortality was determined by placing five fish into each of three paired samples of 500 ml aged wastewater and distilled water containing the same DO, CO_2, and pH for 24 hours. The pH (4.7), CO_2 (80 ppm), and DO (8.0 ppm) were adjusted as previously described.

Minimum pH necessary for fish survival in wastewater was established by placing five fish in 500 ml samples containing same amounts of CO_2 (240 ppm) and DO (0.8 ppm) but with varying pH (4.7, 4.8, 4.9, 5.0, 5.3, 5.5, and 6.0).

Source of Limiting Factors

Source of Acidity and CO_2 in Mill Wastewater. Results of biological decomposition of suspended cane stalk fibers and dissolved sugar in wastewater were observed for changes in pH and CO_2 content. Two sugar cubes (7.5 g) and 7.5 g of cane stalk pulverized in a food blender were each placed in 500 ml of mill wastewater and sampled for pH and CO_2 at 24-hour intervals for 72 hours. Control samples used for comparison contained only wastewater. This test was repeated three times.

Increased pH and CO_2 Caused by Cane Fibers. Substantiation of the previous study was done by filtering suspended particles of cane stalk from wastewater and comparing pH levels and CO_2 production with unfiltered samples. Filtering was done with qualitative filter paper efficient at 11 microns. Five different samples were tested at 24-hour intervals for 72 hours.

RESULTS

Limiting Factors

Mill Wastewater Analyses. Of possible fish toxicants cited by Douderoff (Brown 1957), only CO_2, DO, iron, and pH were considered because data on Table 1 showed large changes in concentrations from those at the mill outfall.

Sulfides and ammonia were discounted as toxicants because concentrations were far below lethal concentrations of 2 ppm sulfides and 18 ppm ammonia cited by Sasa (Laird 1981). Chronic toxicity from other sources were also discounted because *P. reticulatus* were successfully reared in aquariums where half the water was replaced every third day with new wastewater for five months.

Bioassay for DO, CO_2, and pH. Data on Table 2 indicated that low pH was the critical factor of fish mortality. Interference of high CO_2 levels in O_2 utilization by fish (Jones 1964) was apparently not as critical as low pH even at low levels of DO (0.4 ppm).

Bioassay of Low pH in Wastewater. Survival of all fish in distilled water and the deaths of all fish in wastewater samples containing same levels of pH, DO, and CO_2 indicated that another factor was linked to low pH and fish mortality.

TABLE 1. Analyses of Potential Fish Toxicants in Sugar Mill Wastewater.

Potential Toxicants	Sampling Sites		
(Mean ± S.D. n=5)	Mill Outfall	Impoundment Basin	Irrigation Canal
Temperature (°C)	39.60 ± 1.34	27.60 ± 1.14	26.60 ± 0.55
Salinity (%)	0.54 ± 0.08	0.52 ± 0.11	0.50 ± 0.10
pH	6.36 ± 0.15	4.90 ± 0.32	5.08 ± 0.55
Turbidity (cm)	1.27 ± 0.00	6.35 ± 2.18	7.11 ± 1.45
DO Surface (ppm)	—	1.01 ± 0.35	1.03 ± 0.17
15 cm	0.37 ± 0.00	0.29 ± 0.07	0.30 ± 0.11
CO_2 (ppm)	33.80 ± 7.59	190.20 ± 61.28	146.20 ± 70.73
Sulfides (ppm)	<0.2	<0.2	0.41 ± 0.27
Ammonia (ppm)	—	4.33 ± 1.15	3.80 ± 1.09
Iron (ppm)	—	10.25 ± 2.06	9.88 ± 1.75

TABLE 2. Effect of Varying Concentrations and Combinations of DO, CO_2, and pH in Sugar Mill Wastewater on Fish Mortality Rates.

		Test Samples				
	Control	1	2	3	4	5
pH	6.5	6.1	4.7	4.7	4.7	4.7
DO (ppm)	0.4	0.4	0.4	0.4	14.0	11.2
CO_2 (ppm)	80.0	250.0	250.0	80.0	250.0	80.0
Mortality (%)	0	0	100	100	100	100

Results of fish exposed to mill wastewater with different pH levels were as follows: at pH 4.8 and below, all fish died; at pH 4.9, 5.0, and 5.3, 60% survived; and at pH 5.5 and 6.0, all fish survived.

Iron in Wastewater. Samples taken from the wastewater basin and canal showed iron content of 8–12 ppm (Table 1). These concentrations indicated that iron may have been the other factor linked to low pH responsible for fish mortality since they were much higher than the lethal concentrations of 1.2–10.5 ppm at pH 4.3–4.4 cited by Alabaster and Lloyd (1980) for one-year old carp and the 0.41 ppm at pH 5.5 for brook trout found by Decker and Menendez (1974).

Source of Limiting Factors

Source of Acidity and CO_2 in Mill Wastewater. Data on Table 3 showed that biological decomposition of cane fibers was the primary cause of CO_2 production and low pH in sugar mill wastewater. Changes in pH levels and CO_2 concentrations were distinctly greater in cane fiber samples during the first 24 hours.

Increased pH and CO_2 Caused by Cane Fibers. Results of filtration of mill wastewater to remove microscopic particles of cane stalks produced similar changes in pH and CO_2 as in Table 3. Significant changes in pH and CO_2 occurred during the first 24 hours when unfiltered control samples produced 127.1 ± 19.4 ppm of CO_2 (pH 5.0 ± 0.0) and filtered samples 60.2 ± 9.7 ppm CO_2 (pH 5.2 ± 0.1).

TABLE 3. Changes in CO_2 and pH in Sugar Mill Wastewater Caused by Biological Decomposition of Sugar and Cane Fibers.

Test Sample (Mean ± S.D. n=3)		Elapsed Time (hours) 0	24	48	72
7.5 g Sugar/	CO_2 (ppm)	143.0 ± 0.0	264.0 ± 8.2	681.0 ± 81.9	533.66 ± 74.20
500 ml Wastewater	pH	5.0 ± 0.0	4.7 ± 0.0	5.0 ± 0.3	4.4 ± 0.0
7.5 g Cane Fibers/	CO_2	143.0 ± 0.0	476.7 ± 10.0	682.3 ± 54.2	267.7 ± 93.2
500 ml Wastewater	pH	5.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	5.5 ± 0.2
Control					
500 ml Wastewater	CO_2	143.0 ± 0.0	138.7 ± 40.1	131.7 ± 14.2	76.3 ± 12.5
	pH	5.0 ± 0.0	5.3 ± 0.0	5.5 ± 0.0	5.7 ± 0.0

DISCUSSION

Results indicated that acidity levels in sugar mill wastewater must be maintained above pH 4.9 to assure fish survival. There was no tolerance below this acidity level since all fish died regardless of DO and CO_2 levels. My observations of fish surviving in impounded sugar mill wastewater only in areas with acidity above pH 4.9 coincides with results of this study.

Low DO combined with interference of O_2 utilization by high CO_2 levels (Alabaster et al. 1957) was also a factor in fish mortality. However, survival of some fish under conditions of low DO and high CO_2 levels in waters with pH above 4.9 suggested that *P. reticulatus* could be selectively bred to tolerate such conditions. My observations of *P. reticulatus* dying within 15 minutes in cages held below the surface of wastewater (0.3 ppm DO) where they were captured agree with Sasa's (Laird

1981) observations that this species is able to survive in water completely devoid of DO as long as they had access to the water surface.

The cause of iron in toxic amounts as outlined by Ruttner (1963) closely follows the conditions found in sugar mill wastewater (Table 1). Iron, as soluble ferrous bicarbonate, usually is found: (1) when it is nearly free of dissolved oxygen; (2) when it contains adequate amounts of carbon dioxide; (3) when the pH is not above pH 7.5; and (4) when organic substances arising from decomposition are present which can reduce ferric hydroxide.

Wedemeyer et al. (1976) attributed the lethal effects of iron in wastewater to precipitation of ferric hydroxide on fish gills that interfered with the transport of O_2 across the gill epithelium. Their photomicrographs of fish gills coated with precipitated ferric hydroxide were similar in appearance to the gills of fish killed in sugar mill wastewater that I examined.

Other factors that indicated fish mortality was caused by gills clogged with iron precipitated when acidity fell below pH 5.5 were: (1) fish dying sooner in vigorously aerated wastewater (pH 4.7, DO 6.0 ppm) than those in similar unaerated water suggesting that exertions to maintain equilibrium in strong aeration currents caused a more rapid depletion of their O_2 supply; (2) fish unharmed by pH 4.7 in distilled water indicating a relationship between low pH and another substance in mill wastewater as the cause of fish mortality; (3) fish mortality occurring only below pH 5.5 in this study coinciding with Huet's (1972) warning on fish culture that at pH 5.5 and below precipitation of iron on the gills interferes with respiration; (4) the presence of iron in mill wastewater at levels considered toxic at low pH; and (5) the complete recovery of moribund fish when placed in clean tapwater suggesting that a reversible mechanical obstruction, such as precipitated iron, rather than poisons or gill damage caused asphyxiation.

Ruttner's (1964) citing of a study showing soluble ferrous bicarbonate almost instantaneously precipitating to insoluble ferric hydroxide when the O_2 content of water was higher than 0.5 ppm or the pH higher than neutrality suggested three possibilities for the precipitation of iron on fish gills: (1) the alkaline surface of fish gills (Duijn 1967); (2) the presence of O_2 at levels above 0.5 ppm at the surface of fish gills; or (3) the higher O_2 content at the wastewater surface (Table 1) precipitated ferric hydroxide that accumulated on the gills of fish skimming the surface to obtain O_2.

It is apparent from this study that the simplest solution to the toxic effects of iron in wastewater is to maintain the pH above 4.9 by reducing the cause of acidity. Of the two possible causes of acidity, cane fiber appears to be the major cause because of: (1) the improbability of wastewater containing as much sugar as the samples in Table 3 since this would be equivalent to 498 tons of sugar in the eight million gallons of wastewater discharged daily; (2) the rise in pH after removal of cane fiber particles by filtration; and (3) similar fluctuations in acidity as the cane fiber samples in Table 3 of 10 wastewater samples monitored over 72 hours (pH levels: 0 h, 5.61 $\pm$ 0.35; 24 h, 5.03 $\pm$ 0.21; 48 h, 5.19 $\pm$ 0.38; and 72 h, 5.67 $\pm$ 0.29). A major source of these suspended microscopic cane particles is from sludge precipitated by liming during clarification of cane juice. The U.S. Environmental Protection Agency's study on Hawaiian sugar mill waste (1971) states that several tons of mud and fine fibers per day from vacuum filtration of clarified cane juice is combined with wastewater at most plants. Separation of this filter sludge from mill wastewater may reduce pH to tolerable levels for survival of mosquito larvivorous fish in wastewater impoundment basins. The cost of sludge removal, either by trucking or by pumping a slurry to a separate basin, must be weighed against the cost of the mosquito larviciding program for each plantation.

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